Effects of sheep and horse manure and pine bark amendments on metal distribution and chemical properties of contaminated mine soils

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Summary

Mine soils usually contain large levels of heavy metals and poor fertility conditions which limit their reclamation and the application of phyto-remediation technologies. Two organic waste materials (pine bark compost and sheep and horse manure compost), with different pHs and varying degrees of humification and nutrient contents, were applied as amendments to assess their effects on copper (Cu) and zinc (Zn) bioavailability and on fertility conditions of mine soils. Soil samples collected from two abandoned mining areas near Madrid (Spain) were mixed with 0, 30 and 60 t ha^{-1} of the organic amendments. The concentrations of metals among the different mineral and organic fractions of soil were determined by several extraction procedures to study the metal distribution in the solid phase of the soil affected by the organic amendments. The results showed that the manure amendment increased the soil pH and the cation exchange capacity and enhanced the nutrient levels of these soils. The pine bark amendment decreased the soil pH and did not significantly change the nutrient status of soil. Soil pH, organic matter content and its degree of humification, which were altered by the amendments, were the main factors affecting Cu fractionation. Zn fractionation was mainly affected by soil pH. The addition of manure not only improved soil fertility, but also decreased metal bioavailability resulting in a reduction of metal toxicity. Conversely, pine bark amendment increased metal bioavailability. The use of sheep and horse manure could be a cost-effective practice for the restoration of contaminated mine soils.

Introduction

Mine soils usually contain large levels of heavy metal contaminants, even several years after the mine is abandoned. This heavy metal accumulation can be a potential concern for both human and animal health, decreases plant growth and ground cover and increases the risk of metals leaching into groundwater. Moreover, these soils often have poor conditions for plant growth including poor physical structure, sandy texture, acidity, poor cation exchange capacity (CEC) and small organic matter and nutrient contents, which limit the establishment of vegetation and intensify erosion by rain and wind (Tordoff *et al.*, 2000).

Previous work has evaluated the application of organic materials as amendments to reclaim acid mine soils (Walker *et al.*, 2004; Clemente *et al.*, 2006; Frutos *et al.*, 2010). The use of organic amendments can improve the soil structure, water holding capacity (WHC), CEC and nutrient status, which encourage the re-establishment of the vegetation cover on contaminated sites and reduces surface erosion (Tordoff *et al.*, 2000; Mendez & Maier, 2008). Amendments can also reduce the bioavailability of metals and therefore, their toxicity toward plants and other organisms. The application of these organic amendments can redistribute metals from labile (water-soluble and exchangeable) to less available fractions (organic or residual). This reduction in metal bioavailability is the result of increasing sorption of metals onto colloidal particle surfaces, and the formation of stable complexes with humic substances. These substances provide an important number of carboxylic (–COOH), hydroxylic (–OH) and phenolic (aromatic ring–OH) functional groups, which have a large affinity for metals. Sorption depends on the metal concerned, soil type, degree of humification of the organic matter, total metal content, salinity, pH and redox potential (Narwal & Singh, 1998; Shuman, 1999; Walker *et al.*, 2004). Conversely, the addition of relatively new organic amendments such as fresh compost with a large proportion of fulvic acids (FAs) and soluble organic

matter content could increase metal mobility through the formation of soluble organo-metallic complexes (Shuman, 1999; Clemente *et al.*, 2006). Schwab *et al.* (2007) reported an increasing risk of metal leaching and consequent groundwater contamination in the presence of organic amendments because of complex formation between metals and soluble organic matter.

Iron (Fe) and manganese (Mn) oxides also play an important role in metal-retention in soils (Hall *et al.*, 1996). They have a large specific surface area (30–300 $m^2\ g^{-1}$) and – OH functional groups, with a large sorption capacity, which varies with the soil pH. The redox reactions of these compounds and their degree of crystallinity may influence metal mobility.

Although the feasibility of organic amendments for soil remediation has been widely studied, there is little information about their effects on the metal distribution between organic amendment components such as humic acids (HAs) and soil components such as metal oxides. These materials can immobilize metals, improve soil fertility and be used to reclaim abandoned mine soils and to aid future phyto-remediation strategies. The aim of this work was to evaluate the effect of two organic amendments (sheep and horse manure compost and pine bark compost), each with a different pH and degree of humification, on soil fertility and metal bioavailability. The metal concentrations among different mineral and organic fractions of the soil were measured using several extraction procedures to understand the metal distribution in the solid phase of the soil affected by these organic amendments.

Materials and methods

Soil sampling and site description

Two heavy metal-contaminated soils from the Lozoya Valley, located in the north of Madrid (Spain), were selected (Figure 1). The site location of the first soil is the village of Garganta de los Montes (GAR), which is in close proximity to a copper (Cu) mine that was abandoned in 1965. The site location of the second soil is situated in El Cuadron (CUA: Figure 1), which contains an old blende mine that was abandoned in 1862.

The lithology of this area is dominated by magmatic gneisses, and the most abundant minerals are chalcopyrite in paragenesis containing small amounts of pyrite, blende and marcasite. There is also some galena and quartz gangue and trace amounts of arsenopyrite are also present. The soils at these site locations have been classified as humic and dystric cambisols (FAO, 1990), and the vegetation is mainly composed of *Quercus pyrenaica* Willd., *Fraxinus angustifolia* Vahl, wet and temperate grasslands, and tilled pastures (Pastor *et al.*, 2007). Soil samples were collected with a stainless steel scoop within the top 20 cm from three different points around the mine tailings. Samples were homogenized, air-dried and sieved to <2 mm for analysis.

The main properties of these soils (GAR and CUA) are shown in Table 1. Both soils showed characteristics typically found in mine soils: acidic, loamy sand texture (USDA), small salinity and CEC, small content of organic matter content and nutrient status (see total nitrogen in Table 1 and exchangeable cation contents in Table 2) and also contained large concentrations of Fe oxides, mainly in their crystalline forms (goethite, hematite). These soils exhibit pH-dependant surface sorption charges which are dependant on the small clay contents and large concentrations of metal oxides.

Both soils contained total concentrations of Cu that exceed the European Union maximum permitted levels for agricultural soils in the pH range 6–7 (50–140 mg kg^{-1}; Council of the European Communities, 1986). The GAR soil contained larger levels of metals than CUA, especially Cu levels. Kabata-Pendias & Pendias (2001) established the critical total metal concentration for soils, and there is a potential for toxicity in plants at levels greater than this maximum. According to the limits set by Kabata-Pendias & Pendias (2001), the Cu concentration was greater than the upper critical level (60–125 mg kg^{-1}) in both soils. Conversely, the zinc (Zn) levels in the soil were less than the upper toxic level (70–400 mg kg^{-1}).

Organic amendments

Two organic amendments provided by a commercial supplier were used in this study: compost made of sheep (50% v/v) and horse

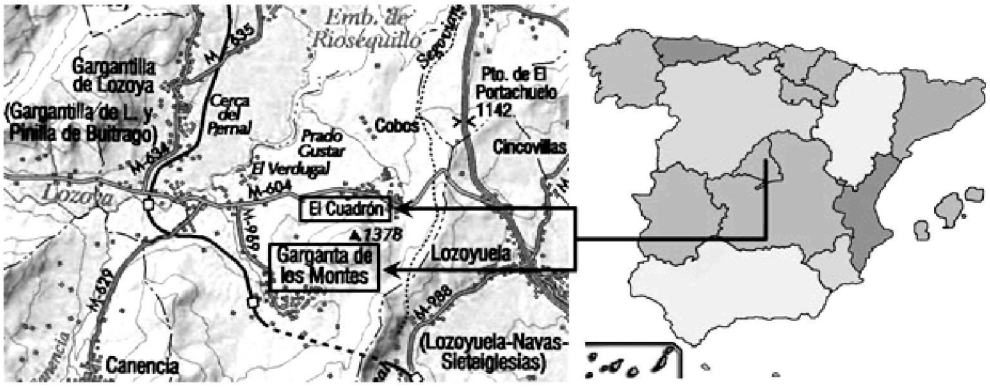

Figure 1 Location of Garganta de los Montes and El Cuadrón (Madrid, Spain).

Table 1 Properties of the soils and the organic amendments

Properties	GAR	CUA	M	P
Sand / %	78.2	79.4	nd	nd
Silt / %	18.8	17.3	nd	nd
Clay / %	3.1	3.3	nd	nd
pH	6.2	5.5	9.4	5.6
EC / dS m^{-1}	0.08	0.10	4.95	0.66
CEC / $cmol_c$ kg^{-1}	4.74	4.79	nd	nd
TOC / %	0.88	1.39	27.2	45.8
OXC / %	0.74	1.37	16.9	41.5
EXC / %	0.20	0.42	5.4	6.2
HA / %	0.07	0.12	2.6	2.1
FA / %	0.13	0.30	2.8	4.1
HR / %	22.5	30.4	19.7	13.4
HI / %	7.7	8.8	9.4	4.6
N / %	0.04	0.09	1.5	0.7
C:N	22	15	19	69
Fe_{asc-ox} / g Fe kg^{-1}	11.9	7.31	3.82	2.27
Fe_{ox} / g Fe kg^{-1}	2.04	1.11	1.72	1.31
Mn_{asc-ox} / g Mn kg^{-1}	0.26	0.28	0.36	0.10
Mn_{ox} / g Mn kg^{-1}	0.16	0.23	0.25	0.05
Total Cu[a] / mg kg^{-1}	913	248	30.9	1.07
Total Zn / mg kg^{-1}	203	146	179	35.7

[a]European Union limits (mg kg^{-1}) for agricultural soils receiving sewage sludge (pH 6–7): Cu 50–140 and Zn 150–300 (Council of the European Communities, 1986).

EC, electrical conductivity; CEC, cation exchange capacity; TOC, total organic carbon; OXC, oxidisable organic carbon; EXC, humic extractable organic carbon; HA, humic acids carbon; FA, fulvic acids carbon; HR, humification ratio; HI, humification index; N, total nitrogen; Fe_{asc-ox} and Mn_{asc-ox}, concentrations of Fe and Mn extracted with the ascorbate–oxalate solution; Fe_{ox} and Mn_{ox}, concentrations of Fe and Mn extracted with the oxalate solution in the dark; GAR, non-amended Garganta soil; CUA, non-amended Cuadron soil; M, sheep and horse manure amendment; P, pine bark amendment; nd, not determined.

(50%) manure (M) and a compost of pine bark (50% v/v), wood fibre (30%) and *Sphagnum* peat (20%) (P). The manure compost is usually applied as soil amendment at 20–40 t ha^{-1} of dry organic matter and it was composted for 4–5 months in heaps with periodic turning in an open-air composting plant located in Segovia (Spain). This amendment has a price of US \$30 m^{-3} (and a density of 800 kg m^{-3}). The pine bark compost is normally used as growing medium in pot culture and it was composted for 9–10 months in heaps under open-air conditions with periodical turning before the composted barks were ground, sieved and mixed with wood fibre and peat. Pine bark compost has a price of US \$41 m^{-3} (density of 300 kg m^{-3}).

Table 1 also shows the main properties of these organic amendments (M and P). The manure amendment had higher pH, salinity (electrical conductivity, EC), total nitrogen (N) content and Fe and Mn oxides than pine bark. The manure also had a greater degree of humification (humification ratio, HR), a greater proportion of humic acids (humification index, HI) and a smaller C:N ratio, which indicate a greater degree of maturity and stability than the pine bark amendment. Manure had larger total metal concentrations, especially Zn, which is probably because of the use of this metal as a nutritional supplement in feed for intensive animal production (Bolan *et al.*, 2004).

Experimental design

Ten different treatments were prepared with mixtures of each soil (GAR and CUA) and one of the amendments (M and P). The applied doses were 0, 30 and 60 t ha^{-1} of dry organic matter. Soils were mixed with 29.7 g (30 t ha^{-1}) and 59.5 g (60 t ha^{-1}) of manure per kg of soil and with 15.1 g (30 t ha^{-1}) and 30.1 g (60 t ha^{-1}) of pine bark per kg of soil. Treatments of soil GAR were as follows: non-amended soil as control (GAR0), soil with 30 t ha^{-1} of manure compost (GAR30M), soil with 60 t ha^{-1} of manure (GAR60M), soil with 30 t ha^{-1} of pine bark compost (GAR30P) and soil with 60 t ha^{-1} of manure (GAR60P). Treatments with soil CUA followed the same sequence: CUA0, CUA30M, CUA60M, CUA30P and CUA60P.

All mixtures were homogenized in a cement mixer with 8 kg of soil and the corresponding quantity of amendment. The mixtures (2 kg) were placed in trays in the laboratory and were moistened with deionised water to 60% of their WHC (92 g kg^{-1} in soil GAR and 76 g kg^{-1} in soil CUA) and incubated at room temperature for 4 weeks. The mixtures were mixed daily during the incubation, maintaining the moisture content. Samples were again homogenized, air-dried and stored for analyses. A small amount of sample was stored at 4°C for the determination of the water-soluble organic carbon (C) content.

Analytical methods

All reagents used were of analytical grade or better. Double deionized water was used for all dilutions, and all plastic and glassware was previously soaked in a 5% HNO_3 solution over night and rinsed with distilled water before use.

Soils, amendments and the prepared mixtures were analysed for total organic carbon (TOC) by loss-on-ignition (16 hours at 400°C for soils and 6 hours at 450°C for amendments) and analysed for oxidizable organic carbon (OXC) using a modified Walkley–Black procedure (Nelson & Sommers, 1996). Total N was determined by Kjeldahl digestion (Bremner, 1996). EC and pH were analysed in deionized water extracts (1:2.5 w/w for soils and mixtures; 1:5 v/v for amendments). CEC and exchangeable cations, such as calcium (Ca), magnesium (Mg) and potassium (K), were determined with the barium chloride method (Rhoades, 1982). Texture was determined using the Bouyoucos hydrometer method (Day, 1965).

Samples were extracted three times for 30 minutes at 95° C using a 0.1 M ascorbic acid, 0.2 M oxalic acid and 0.2 M ammonium oxalate solution (1:40 w/v) at pH 3. This ascorbate–oxalate solution is able to dissolve Fe and Mn from amorphous and crystalline oxides in a similar amount to that obtained with sodium dithionite (Shuman, 1982). Samples were also extracted with a

Table 2 Chemical properties of the different mixtures of soils with organic amendments[a]

Treatment	pH	EC / dS m^{-1}	CEC / $cmol_c$ kg^{-1}	Exch. Mg / $cmol_c$ kg^{-1}	Exch. Ca / $cmol_c$ kg^{-1}	Exch. K / $cmol_c$ kg^{-1}
GAR0	6.2 ± 0.1	0.08 ± 0.01	4.74 ± 0.05	0.50 (*0.18*) ± 0.00	2.31 ± 0.07	0.25 (*0.10*) ± 0.00
GAR30M	6.5 ± 0.1	0.51 ± 0.03	5.28 ± 0.10	0.76 (*0.24*) ± 0.03	2.73 ± 0.06	1.18 (*0.34*) ± 0.08
GAR60M	6.8 ± 0.1	0.94 ± 0.01	5.99 ± 0.20	1.00 (*0.30*) ± 0.07	3.44 ± 0.17	2.24 (*0.51*) ± 0.11
GAR30P	5.9 ± 0.0	0.11 ± 0.00	5.30 ± 0.12	0.59 (*0.20*) ± 0.03	2.57 ± 0.05	0.28 (*0.11*) ± 0.02
GAR60P	5.6 ± 0.0	0.17 ± 0.00	5.68 ± 0.42	0.63 (*0.21*) ± 0.01	2.62 ± 0.04	0.30 (*0.11*) ± 0.01
F^b	79.9	624.3	4.6	31.6	22.2	333.9
P	<0.001	<0.001	0.023	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.173	0.058	0.691	(*0.026*)	0.282	(*0.032*)
CUA0	5.6 ± 0.0	0.10 (*0.02*) ± 0.00	4.79 ± 0.10	0.61 (*0.21*) ± 0.01	3.28 ± 0.04	0.66 (*0.22*) ± 0.02
CUA30M	5.8 ± 0.0	0.58 (*0.76*) ± 0.03	5.67 ± 0.21	0.93 (*0.29*) ± 0.08	4.00 ± 0.21	2.00 (*0.48*) ± 0.08
CUA60M	6.1 ± 0.0	0.99 (*0.99*) ± 0.04	6.16 ± 0.11	1.09 (*0.32*) ± 0.04	4.09 ± 0.14	3.08 (*0.61*) ± 0.07
CUA30P	5.4 ± 0.0	0.16 (*0.20*) ± 0.00	4.87 ± 0.11	0.64 (*0.22*) ± 0.02	3.16 ± 0.07	0.72 (*0.24*) ± 0.02
CUA60P	5.3 ± 0.0	0.16 (*0.21*) ± 0.00	5.89 ± 0.66	0.64 (*0.21*) ± 0.03	3.38 ± 0.07	0.74 (*0.24*) ± 0.00
F	107.3	885.2	3.7	24.9	12.6	681.1
P	<0.001	<0.001	0.043	<0.001	0.001	<0.001
$LSD_{0.05}$	0.100	(*0.045*)	1.009	(*0.032*)	0.382	(*0.018*)

[a]Values are means ± SE, $n = 3$. Values in italics and given in parenthesis are log-transformed means and $LSD_{0.05}$ (least significant difference at $P < 0.05$).
[b]F ratio and P (significance level) in ANOVA tests.
EC, electrical conductivity; CEC, cation exchange capacity, Exch. Mg, exchangeable magnesium; Exch. Ca, exchangeable calcium; Exch. K, exchangeable potassium; GAR0, non-amended Garganta soil; GAR30M and GAR60M, Garganta soil mixed with 30 and 60 Mg ha^{-1} of manure, respectively; GAR30P and GAR60P, Garganta with 30 and 60 Mg ha^{-1} of pine bark; CUA0, CUA30M, CUA60M, CUA30P and CUA60P, the same sequence for Cuadron soil.

0.2 M ammonium oxalate and 0.2 M oxalic acid solution (1:60 w/v) at pH 3 for 2 hours at room temperature in the dark (Smith & Mitchell, 1987). The oxalate solution in the dark dissolves mainly amorphous or poorly crystalline forms of Fe and Mn oxides. The concentrations of Fe and Mn were measured in these extracts to determine the metal oxide content with different degree of crystallinity.

The water-soluble carbon (WSC) in the samples was extracted in a 1:10 (w/v) ratio and digested with potassium dichromate and sulphuric acid at 160°C for 10 minutes (Ciavatta *et al.*, 1991). A spectrophotometric method was used to measure the concentration of Cr^{3+} produced by the reduction of Cr^{6+} ($\lambda = 590$ nm; Benito *et al.*, 2009).

The humic-extractable organic carbon (EXC) in the samples was determined by using 0.1 M sodium hydroxide and 0.1 M sodium pyrophosphate solution (1:20 w/v) at pH 13. The FA carbon content was measured after the precipitation of HA at pH 2 in the previous extract. The C content was measured using the same method described for the OXC. HR was calculated as: HR = (EXC/TOC) × 100. The HI was calculated as: HI = (HA/TOC) × 100 (Benito *et al.*, 2009).

The total Cu and Zn contents in the soil samples were determined after microwave-assisted acid digestion of the soils with *aqua regia* (International Standards Organization, 1995). One gram of an air-dried ground sample was placed in a Teflon vessel with 2.35 ml 65% HNO_3 and 7 ml 37% HCl. The vessel was placed in a microwave and the temperature maintained at 190°C for 25 minutes. After the vessel was cooled, the solution was filtered and diluted to 50 ml with deionized water. Organic amendments were digested by placing 0.25 g sample in a vessel with 2 ml HNO_3 65%, 6 ml HCl 37% and 1 ml H_2O_2 30% in the microwave at 190°C for 25 minutes (US Environmental Protection Agency, 1996). The solution was filtered after cooling and diluted to 50 ml.

Metal fractionation of soil and mixture samples was carried out with the sequential extraction procedure of Tessier *et al.* (1979) as described in Ma & Rao (1997). The only modification of this procedure was that the residual fraction was digested with HNO_3–HCl mixture. One gram of each sample was placed into a 40 ml centrifuge tube and the following operationally-defined fractions were obtained: water-soluble, exchangeable, acid-soluble, bound-to-Fe–Mn oxides, bound-to-organic-matter-and-sulphides and residual. The water-soluble fraction was extracted with 15 ml of deionized water for 2 hours. The exchangeable fraction was extracted with 8 ml of 1 M $MgCl_2$ (pH 7) for 1 hour. The acid-soluble fraction was extracted with 8 ml of 1 M NaOAc (pH 5) for 5 hours. The bound-to-Fe–Mn oxide fraction was extracted with 20 ml of 0.04 M $NH_2OH{\cdot}HCl$ in 25% (v/v) HOAc at 96°C with occasional agitation over 6 hours. The bound-to-organic-matter-and-sulphides fraction was extracted with 3 ml 0.02 M HNO_3 and 5 ml 30% H_2O_2 (pH 2) at 85°C with occasional agitation for 2 hours; a second extraction was performed with 3 ml of 30% H_2O_2 (pH 2) for 3 hours at 85°C with occasional agitation, and after cooling, 5 ml 3.2 M NH_4OAc in 20% (v/v) HNO_3 was added, the sample was diluted to 20 ml and continuously agitated for 30 minutes at room temperature. The residual fraction was digested with HNO_3–HCl mixture.

The distribution of metals in the humic fractions was determined by measuring their concentrations in the FA and EXC extracts according to the extraction scheme developed by Krishnamurti & Naidu (2000). Metal concentrations were measured in sodium hydroxide and sodium pyrophosphate solution (EXC) to determine the metal–organic complex-bound fraction. After acidification of the previous solution to pH 2 (FA), the metals solubilized by acid, which may contain metals associated with both humic and FA fraction, were determined in the supernatant and designated as a FA-complex-bound fraction. The difference between the metal concentrations in both fractions (EXC and FA) corresponded to the metals strongly bound to HA which resist desorption at pH 2 and was termed HA-complex-bound fraction.

Metal concentrations were measured in the ascorbate–oxalate solution and in the oxalate solution in the dark to determine the amount of metals associated with crystalline and/or amorphous metal oxides.

Metal concentrations in the extracts were determined by atomic absorption spectrophotometry. All analyses were performed in triplicate, with three samples from each treatment tray after its incubation. Values were adjusted to an oven-dried (over night at 105°C) soil basis.

Statistical analyses

Statistical treatment of the experimental data was conducted using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Standard errors (SEs) were calculated to determine the variability of sample means between replicates. The normal distribution of data was checked using the Shapiro–-Wilk's test and the homogeneity of variance was checked using Levene's test. If necessary, the values were log-transformed. Data were tested by one-way analysis of variance (ANOVA) to explore the effects of the organic amendments. Treatment means were compared by the least significant difference test at a significance level of $P < 0.05$. The relationships between different characteristics were determined with Pearson's correlation coefficients (r) using a two-tailed test.

Results and discussion

Effects of organic amendments on soil chemical properties

Table 2 shows the main chemical properties of the different mixtures of mine soils (GAR and CUA) with organic amendments (M and P). Increasing addition of manure significantly raised the pH of both soils, which could reduce metal availability, while the low pH of pine bark reduced the pH of the mixtures ($P < 0.05$). Manure amendment also significantly increased the EC of the soils ($P < 0.05$).

Additions of 60 t ha^{-1} of manure and pine bark amendments caused a significant increase in CEC ($P < 0.05$), which can increase metal sorption. The number of exchange sites increased because of the ionization of –COOH groups and phenolic–OH groups of the organic matter. Significant correlations were found between CEC and OXC ($r = 0.89$ for GAR treatments; $r = 0.94$ for CUA treatments; $P < 0.05$; $n = 5$).

The manure amendment not only raised CEC, provided organic matter and an improved pH of the soil, but also significantly increased exchangeable Ca^{2+}, Mg^{2+} and K^{+} extracted by $BaCl_2$

Table 3 Carbon content in the different mixtures of soils with organic amendments[a]

Treatment	OXC / %	TOC / %	EXC / %	HA / %	FA / %	WSC / mg C kg^{-1}
GAR0	0.7 ± 0.1	0.9 ± 0.0	0.20 ± 0.08	0.07 ± 0.01	0.13 ± 0.07	100 (*2.01*) ± 7.2
GAR30M	1.2 ± 0.0	1.1 ± 0.0	0.22 ± 0.03	0.07 ± 0.03	0.15 ± 0.06	188 (*2.28*) ± 26
GAR60M	1.3 ± 0.1	1.5 ± 0.0	0.32 ± 0.08	0.08 ± 0.01	0.23 ± 0.09	284 (*2.46*) ± 70
GAR30P	0.9 ± 0.1	1.1 ± 0.1	0.28 ± 0.05	0.11 ± 0.06	0.17 ± 0.04	166 (*2.22*) ± 44
GAR60P	1.2 ± 0.1	1.5 ± 0.0	0.48 ± 0.02	0.21 ± 0.02	0.27 ± 0.02	187 (*2.27*) ± 19
F^b	8.8	62.7	3.8	3.6	0.9	3.9
P	0.003	<0.001	0.039	0.047	0.496	0.037
$LSD_{0.05}$	0.257	0.101	0.182	0.100	0.199	(*0.251*)
CUA0	1.4 ± 0.0	1.4 ± 0.0	0.42 ± 0.05	0.12 ± 0.03	0.30 ± 0.02	107 ± 19
CUA30M	1.7 ± 0.0	1.6 ± 0.0	0.48 ± 0.03	0.17 ± 0.11	0.28 ± 0.08	172 ± 7.2
CUA60M	2.0 ± 0.1	1.9 ± 0.0	0.55 ± 0.07	0.17 ± 0.11	0.43 ± 0.08	244 ± 25
CUA30P	1.6 ± 0.1	1.6 ± 0.0	0.79 ± 0.01	0.24 ± 0.03	0.54 ± 0.02	136 ± 25
CUA60P	1.7 ± 0.0	1.7 ± 0.0	0.87 ± 0.05	0.24 ± 0.01	0.63 ± 0.04	157 ± 33
F	46.9	46.0	18.0	1.1	8.5	4.9
P	<0.001	<0.001	<0.001	0.434	0.004	0.02
$LSD_{0.05}$	0.100	0.081	0.141	0.169	0.154	73.45

[a]Values are means ± SE, $n = 3$. Values in italics and given in parenthesis are log-transformed means and $LSD_{0.05}$ (least significant difference at $P < 0.05$).
[b]F ratio and P (significance level) in ANOVA tests.
OXC, oxidizable organic carbon; TOC, total organic carbon; EXC, humic extractable organic carbon; HA, humic acids carbon; FA, fulvic acids carbon; WSC, water-soluble organic carbon; GAR0, non-amended Garganta soil; GAR30M and GAR60M, Garganta soil mixed with 30 and 60 Mg ha^{-1} of manure, respectively; GAR30P and GAR60P, Garganta with 30 and 60 Mg ha^{-1} of pine bark; CUA0, CUA30M, CUA60M, CUA30P and CUA60P, the same sequence for Cuadron soil.

Table 4 Concentrations of Cu (mg kg^{-1}) among the fractions in the different mixtures of soils with organic amendments obtained by a sequential extraction procedure[a]

Treatment	Water-soluble	Exchangeable	Acid-soluble	Fe–Mn oxides	Organic	Residual	Sum of fractions
GAR0	3.1 ± 0.1	27 ± 1.2	90 ± 2.3	397 ± 5.6	192 ± 24	127 ± 12	836 ± 39
GAR30M	4.5 ± 0.2	19 ± 1.3	80 ± 2.8	356 ± 12	276 ± 26	129 ± 7.8	865 ± 42
GAR60M	4.8 ± 0.4	16 ± 3.2	64 ± 3.1	335 ± 12	262 ± 23	120 ± 10	802 ± 26
GAR30P	4.7 ± 0.1	34 ± 1.9	86 ± 2.0	367 ± 2.7	232 ± 18	145 ± 23	869 ± 47
GAR60P	4.1 ± 0.3	34 ± 3.0	81 ± 1.2	352 ± 7.7	248 ± 29	130 ± 9.0	850 ± 40
F[b]	7.7	13.2	17.0	7.1	1.8	0.5	0.5
P	0.001	<0.001	<0.001	0.002	0.191	0.738	0.729
$LSD_{0.05}$	0.741	6.880	7.160	26.06	72.36	35.18	114.9
CUA0	2.0 ± 0.3	10 ± 0.3	27 ± 0.6	66 ± 1.2	49 ± 2.0	56 ± 1.6	211 ± 2.7
CUA30M	1.9 ± 0.2	4.6 ± 0.3	19 ± 0.5	57 ± 0.6	63 ± 2.6	54 ± 4.4	199 ± 6.9
CUA60M	2.2 ± 0.1	1.3 ± 0.2	11 ± 0.1	46 ± 1.1	83 ± 3.9	58 ± 0.5	202 ± 3.2
CUA30P	1.4 ± 0.1	7.6 ± 0.7	22 ± 0.5	59 ± 1.5	60 ± 3.9	51 ± 0.5	201 ± 5.1
CUA60P	1.5 ± 0.1	10 ± 1.3	22 ± 0.3	58 ± 2.3	60 ± 2.1	62 ± 2.8	214 ± 1.6
F	3.3	31.3	184.7	23.7	18.0	2.5	1.2
P	0.041	<0.001	<0.001	<0.001	<0.001	0.086	0.346
$LSD_{0.05}$	0.562	2.036	1.325	4.383	8.809	7.721	13.15

[a]Values are means ± SE, $n = 3$. Values in italics and given in parenthesis are log transformed means and $LSD_{0.05}$ (least significant difference at $P < 0.05$).
[b]F ratio and P (significance level) in ANOVA tests.
GAR0, non-amended Garganta soil; GAR30M and GAR60M, Garganta soil mixed with 30 and 60 Mg ha^{-1} of manure, respectively; GAR30P and GAR60P, Garganta with 30 and 60 Mg ha^{-1} of pine bark; CUA0, CUA30M, CUA60M, CUA30P and CUA60P, the same sequence for Cuadron soil.

($P < 0.05$). The pine bark amendment did not impact significantly on these exchangeable cations in these soils. Organic matter provided by manure acts as a nutrient pool, enhancing nutrient cycling, increases CEC and buffer capacity and improves the physical properties of the soil (Stewart *et al.*, 2000).

Although both amendments raised the TOC and OXC of the soils significantly ($P < 0.05$), the pine bark compost was the only amendment that significantly increased the EXC ($P < 0.05$) (Table 3). This increase in C content in the humic fraction was mainly reflected in a greater proportion of FAs ($P < 0.05$). These compounds are more soluble than HA and could increase metal mobility because of the formation of soluble organo-metallic complexes. The manure amendment did not significantly increase the C content of the humic fractions of the soil.

Although the manure amendment had a small FA content, the WSC was increased significantly by the addition of large doses of this amendment ($P < 0.05$), probably because of its high pH, which reduced the sorption of organic compounds on Fe and Mn oxides and other soil surfaces (Kalbitz *et al.*, 2000). However, no significant correlation was found between soil pH and WSC. An increase in the WSC could result in an increase in metal mobility through the formation of soluble complexes (Zhou & Wong, 2001).

Metal fractionation by a sequential extraction procedure

The total metal concentration cannot be used to assess the environmental impact of contaminated soils, as it is the form of the chemical that determines metal behaviour in the environment and its availability in soils (Ma & Rao, 1997). Table 4 shows the concentrations of Cu among the defined fractions in each treatment obtained using the sequential extraction procedure of Tessier *et al.* (1979). Cu was mostly concentrated in the less labile fractions (Fe and Mn oxide-bound, organic-bound and residual). The Cu concentration in the water-soluble and exchangeable fractions was very small with, therefore, only limited bioavailability in these soils. Cu bioavailability was greater in soil CUA, which had a greater proportion of this metal in the water-soluble (0.7–1.1%) and exchangeable (2.3–4.8%) fractions than GAR (0.4–0.6% water-soluble Cu, 3.3–4.0% exchangeable Cu). This greater bioavailability most probably resulted from the lower pH of soil CUA than in GAR.

The organic matter provided by the amendments and the modification of soil pH contributed to a re distribution of Cu among the different fractions. Previous studies have reported that the modification of organic matter content and soil pH changed Cu fractionation, (Lair *et al.*, 2007). The manure amendment significantly decreased the concentrations of Cu in the exchangeable, acid-soluble and Fe and Mn oxide fractions ($P < 0.05$), while the concentration of Cu in the organic fraction was increased in soil CUA ($P < 0.05$), which reduced the bioavailability of this metal. Previous work has reported similar results with manure amendments (Liu *et al.*, 2003).

The addition of pine bark also increased the Cu concentration in the organic fraction of soil CUA ($P < 0.05$), but this increase was mainly associated with a decrease in the Fe–Mn oxide and acid-soluble fractions ($P < 0.05$) and not with the exchangeable fraction. Pine bark increased the concentration of

Table 5 Concentrations of Zn (mg kg^{-1}) among the fractions in the different mixtures of soils with organic amendments obtained by a sequential extraction procedure[a]

Treatment	Water-soluble	Exchangeable	Acid-soluble	Fe–Mn oxides	Organic	Residual	Sum of fractions
GAR0	0.7 (*0.23*) ± 0.0	6.5 ± 0.3	5.4 (*0.81*) ± 0.2	51 ± 0.9	9.1 (*1.00*) ± 0.7	136 ± 0.9	210 ± 1.1
GAR30M	0.9 (*0.27*) ± 0.0	8.3 ± 0.5	6.1 (*0.85*) ± 0.6	48 ± 2.0	9.3 (*1.01*) ± 0.4	135 ± 3.2	208 ± 4.6
GAR60M	1.0 (*0.31*) ± 0.2	7.9 ± 0.9	5.9 (*0.84*) ± 0.9	47 ± 1.1	9.1 (*1.00*) ± 0.2	130 ± 1.9	201 ± 1.6
GAR30P	1.2 (*0.35*) ± 0.1	8.2 ± 0.4	4.7 (*0.75*) ± 0.1	48 ± 1.1	9.8 (*1.03*) ± 0.6	131 ± 5.9	204 ± 7.0
GAR60P	1.1 (*0.32*) ± 0.1	8.5 ± 0.3	4.9 (*0.77*) ± 0.2	47 ± 1.0	11 (*1.07*) ± 0.8	132 ± 2.7	204 ± 4.3
F[b]	2.5	2.1	1.5	2.0	1.3	0.7	0.8
P	0.090	0.135	0.252	0.143	0.318	0.588	0.532
$LSD_{0.05}$	(*0.082*)	1.672	(*0.095*)	3.910	(*0.067*)	9.137	12.01
CUA0	1.3 ± 0.2	19 ± 1.3	5.0 (*0.78*) ± 0.2	29 ± 1.0	7.9 ± 0.4	59 ± 1.2	120 ± 1.4
CUA30M	1.4 ± 0.2	19 ± 0.7	6.2 (*0.86*) ± 0.6	29 ± 1.3	8.0 ± 0.5	58 ± 6.6	121 ± 9.0
CUA60M	1.2 ± 0.1	15 ± 0.5	7.5 (*0.93*) ± 0.5	33 ± 0.8	8.9 ± 0.2	56 ± 1.3	122 ± 1.4
CUA30P	1.4 ± 0.2	18 ± 0.4	4.9 (*0.77*) ± 0.2	27 ± 0.6	8.0 ± 0.2	59 ± 3.5	119 ± 2.6
CUA60P	1.4 ± 0.1	19 ± 1.7	4.2 (*0.72*) ± 0.1	28 ± 1.1	8.6 ± 0.4	61 ± 2.0	122 ± 2.8
F	0.4	2.5	12.6	4.9	1.6	0.2	0.1
P	0.800	0.085	<0.001	0.010	0.233	0.926	0.966
$LSD_{0.05}$	0.433	3.142	(*0.062*)	2.362	1.121	10.688	13.67

[a]Values are means ± SE, $n = 3$. Values in italics and given in parenthesis are log transformed means and $LSD_{0.05}$ (least significant difference at $P < 0.05$).
[b]F ratio and P (significance level) in ANOVA tests.
GAR0, non-amended Garganta soil; GAR30M and GAR60M, Garganta soil mixed with 30 and 60 Mg ha^{-1} of manure, respectively; GAR30P and GAR60P, Garganta with 30 and 60 Mg ha^{-1} of pine bark; CUA0, CUA30M, CUA60M, CUA30P and CUA60P, the same sequence for Cuadron soil.

Cu in the exchangeable fraction in soil GAR ($P < 0.05$), with reduced bioavailability because of the low pH of this amendment. The results from other studies have shown that pine bark compost has less capacity to immobilize Cd than spent mushroom and pruning waste + biosolids composts because of lower pH, Fe oxides content, degree of humification and CEC (Eymar *et al.*, 2009; Tapia *et al.*, 2010).

The high pH of the manure amendment increased metal sorption on the solid phase of these variable-charged soils. Metal concentrations in the acid-soluble (carbonate-bound and specifically adsorbed metals) and Fe and Mn oxide fractions should increase with an increase in pH, but the concentration of Cu in these fractions decreased when manure was applied ($P < 0.05$). This shows that the pH had a smaller influence on Cu immobilization than the organic matter content. Strong and negative correlations were found between OXC and Cu concentrations in the acid-soluble fraction ($r = -0.89$ for GAR treatments; $r = -0.94$ for CUA treatments; $P < 0.01$; $n = 5$) and the Fe and Mn oxide fraction ($r = -0.96$ for GAR treatments; $r = -0.94$ for CUA treatments; $P < 0.01$; $n = 5$), and there was a positive correlation with Cu concentrations in the organic fraction ($r = 0.89$ for GAR treatments; $r = 0.92$ for CUA treatments; $P < 0.01$; $n = 5$). However, OXC was not correlated with the exchangeable fraction, but a significant and negative correlation was found between this fraction and soil pH ($r = -0.96$ for GAR treatments; $r = -0.94$ of CUA treatments; $P < 0.01$; $n = 5$). McBride *et al.* (1997) also found significant correlations between Cu activity in soil solution and organic matter content, suggesting that the Cu solubility is controlled by sorption on organic matter.

However, the concentration of water-soluble Cu, the most labile fraction, increased when the amendments were added to GAR soil ($P < 0.05$). Although the manure amendment had a good metal-retention capacity, it dissolved a small amount of Cu in the soil because of a large WSC. However, there was no significant correlation found between WSC and the water-soluble Cu. Zhou & Wong (2001) reported that the application to soil of dissolved organic matter from sludge reduced Cu sorption.

The residual Cu in the less labile fraction was not affected by the application of organic amendments. The sum of Cu concentrations in all fractions was similar to the total Cu concentration found in these soils and did not significantly vary among the different treatments because of the small amount of Cu provided by the two amendments.

The distribution of Zn among the different fractions in each treatment is shown in Table 5. The majority of Zn was associated with the Fe and Mn oxide and residual fractions in all treatments. The other fractions were of minor importance in these soils. Similar results were reported in previous work (Ma & Rao, 1997; Narwal & Singh, 1998). Despite its smaller total Zn concentration, the concentration of exchangeable Zn in CUA soil was relatively large and greater than that found in GAR soil and had therefore a greater Zn bioavailability, probably because of a lower pH, Fe oxide content and the degree of crystallinity of these compounds.

There was no significant variation found in the Zn distribution among the different fractions of the GAR samples. However, the addition of a large amount of manure to soil CUA decreased the concentration of Zn in the exchangeable fraction and increased the amount of Zn in the acid-soluble and Fe–Mn oxide fractions

($P < 0.05$). This reduction in Zn availability resulted from the high pH of this amendment, which increased Zn sorption on Fe and Mn oxides and other soil particles. In contrast, the addition of the pine bark amendment, with a lower pH, resulted in a slight decrease in the concentration of Zn in the acid-soluble and Fe and Mn oxide fractions ($P < 0.05$).

The application of organic materials did not increase the amount of Zn associated with the organic fraction, whereas the opposite trend was observed for Cu. Zn was more affected by pH-dependant sorption processes than by complexing with organic compounds. These results agree with those reported by Shuman (1999), who observed that the addition of organic materials with high pH values re-distributed Zn from the exchangeable fraction to the Fe–Mn oxide fraction. Narwal & Singh (1998) reported that the organic matter provided by different manure amendments did not affect Zn fractionation in soil. A significant and negative correlation was found between pH and water-soluble Zn fraction in soil CUA ($r = -0.90$; $P < 0.05$; $n = 5$), whereas pH was correlated positively with Zn concentration in the acid-soluble fraction ($r = 0.99$; $P < 0.01$; $n = 5$) and in the Fe and Mn oxide fraction ($r = 0.95$; $P < 0.01$; $n = 5$). However, exchangeable Zn was not correlated significantly with soil pH. Moreover, there were no significant correlations between the organic carbon content and Zn concentration in the different fractions, which showed the limited impact of organic matter on Zn fractionation. Strong and positive correlations were found in soil GAR treatments between the concentrations of Zn bound to Fe and Mn oxides and the concentrations of Fe ($r = 0.97$; $P < 0.01$; $n = 5$) and Mn extracted with oxalate ($r = 0.95$; $P < 0.01$; $n = 5$). These Fe and Mn contents are mainly amorphous oxides, which have a great affinity for Zn. The residual Zn and the sum of all fractions did not significantly vary among the different treatments.

Distribution of metals in the humic fractions

The greater retention of Cu by the organic matter provided by the manure amendment in comparison with the pine bark amendment probably also resulted from the greater degree of humification and proportion of HA. This greater humification results in decreasing metal mobility and availability by sorption and the formation of stable complexes with humic substances (Halim *et al.*, 2003; Clemente *et al.*, 2006; Benito *et al.*, 2009).

Table 6 shows the Cu concentration associated with the metal–organic complex-bound fraction (Cu_{MO}), the proportion of this extracted in the HA-complex-bound fraction (Cu_{HA}) and the FA-complex-bound fraction (Cu_{FA}). The concentration of Cu_{MO} increased significantly with addition of manure in soil GAR ($P < 0.05$), and a greater proportion of Cu was associated with HA (Cu_{HA}) in manure treatments of both soils. The pine bark amendment, which has smaller degree of humification and maturity, did

Table 6 Concentrations of Cu and Zn associated with the humic fractions and with Fe and Mn oxides in the different mixtures of soils with organic amendments[a]

Treatment	Cu_{MO} / mg kg^{-1} soil	Cu_{FA} / %	Cu_{HA} / %	Cu_{asc-ox} / mg kg^{-1} soil	Cu_{ox} / mg kg^{-1} soil	Zn_{MO} / mg kg^{-1} soil	Zn_{FA} / %	Zn_{HA} / %	Zn_{asc-ox} / mg kg^{-1} soil	Zn_{ox} / mg kg^{-1} soil
GAR0	44.6 ± 1.6	100	0	617 ± 13	205 ± 5.3	4.8 ± 0.4	100	0	98.5 ± 1.0	20.0 ± 1.0
GAR30M	56.5 ± 2.9	100	0	601 ± 22	200 ± 10	6.3 ± 1.2	100	0	92.4 ± 2.5	24.9 ± 1.2
GAR60M	62.3 ± 2.4	91	9	570 ± 5.8	182 ± 1.3	7.3 ± 1.2	100	0	92.7 ± 2.8	22.2 ± 0.5
GAR30P	45.5 ± 1.8	100	0	604 ± 9.3	189 ± 4.8	3.7 ± 0.4	100	0	93.4 ± 3.1	19.1 ± 0.6
GAR60P	45.8 ± 2.2	100	0	622 ± 8.8	179 ± 6.5	5.8 ± 1.7	100	0	93.4 ± 1.7	19.0 ± 1.3
F[b]	13.2			2.5	3.0		1.6		1.1	6.6
P	0.001			0.111	0.071		0.262		0.394	0.007
$LSD_{0.05}$	6.929			40.73	19.79		3.513		7.436	3.078
CUA0	97.8 ± 2.5	87	13	175 ± 0.4	122 ± 1.2	27.7 ± 1.3	100	0	94.4 ± 1.3	45.3 ± 1.4
CUA30M	102 ± 5.3	85	15	169 ± 4.1	114 ± 2.3	29.1 ± 1.7	100	0	91.6 ± 1.8	42.7 ± 1.0
CUA60M	98.8 ± 7.0	80	20	164 ± 4.6	110 ± 1.7	30.1 ± 2.2	100	0	90.6 ± 0.8	42.3 ± 0.7
CUA30P	103 ± 11	87	13	171 ± 4.0	118 ± 3.1	28.8 ± 1.7	100	0	93.3 ± 2.4	43.4 ± 0.9
CUA60P	105 ± 8.6	90	10	163 ± 3.0	124 ± 0.1	31.2 ± 2.6	100	0	88.8 ± 3.0	44.8 ± 0.4
F	0.2			2.1	8.8	0.5			1.2	1.9
P	0.958			0.157	0.003	0.757			0.377	0.190
$LSD_{0.05}$	23.37			11.20	6.168	6.124			6.416	2.981

[a]Values are means ± SE, $n = 3$. Values in italics and given in parenthesis are log transformed means and $LSD_{0.05}$ (least significant difference at $P < 0.05$).
[b]F ratio and P (significance level) in ANOVA tests.
Cu/Zn_{MO}, concentrations of metal in the metal–organic complex-bound fraction extracted with a sodium hydroxide and sodium pyrophosphate solution; Cu/Zn_{HA}, proportions of metal associated with the humic acid-complex fraction with respect to the Cu/Zn_{MO}; Cu/Zn_{FA}, proportions of metal associated with the fulvic acid-complex fraction extracted after the acidification to pH 2 of the sodium hydroxide and sodium pyrophosphate solution; Cu/Zn_{asc-ox}, concentrations of metal extracted with the ascorbate–oxalate solution; Cu/Zn_{ox}, concentrations of metal extracted with the oxalate solution in the dark; GAR0, non-amended Garganta soil; GAR30M and GAR60M, Garganta soil mixed with 30 and 60 Mg ha^{-1} of manure, respectively; GAR30P and GAR60P, Garganta with 30 and 60 Mg ha^{-1} of pine bark; CUA0, CUA30M, CUA60M, CUA30P and CUA60P, the same sequence for Cuadron soil.

not increase the Cu_{MO} concentration and the proportion of Cu_{HA}. Hsu & Lo (2001) found that the concentration of Cu associated with the HA fraction in a compost increased with maturity, and 99–100% of Cu was retained in the FA fraction of a fresh compost. Mohamed *et al.* (2010) also reported a greater amount of Cu bound to HA when several manures were applied to soils. Soil CUA contained a greater amount of both Cu_{MO} and Cu_{HA} than soil GAR, which contained less organic matter.

Table 6 also shows the concentrations of Zn associated with the different humic fractions. As reported earlier, the Zn bound to the organic fraction was not affected by the addition of amendments to these soils. There were no significant changes in the concentration of Zn in the metal–organic complex-bound fraction (Zn_{MO}) among the different treatments with organic amendments. In all the treatments, 100% of the Zn_{MO} was associated with the FA-complex fraction (Zn_{FA}). Other authors have also reported a predominance of metals bounded to the FA fraction (Donisa *et al.*, 2003). The greater solubility of FAs and their large numbers of functional groups capable of forming soluble complexes with metals indicate the relatively large mobility of metals bound to the organic fractions of these samples.

Distribution of metals in the Fe and Mn oxide fractions

Table 6 also shows the concentration of Cu extracted with the ascorbate–oxalate (Cu_{asc-ox}) and the oxalate (Cu_{ox}) solutions. The former releases metals from amorphous and crystalline oxides and the latter mainly from amorphous oxides. Both extractions also include more labile fractions, such as water-soluble, exchangeable and acid-soluble, that have not been separated from the oxide fraction, so they cannot be interpreted as exclusively bound to metal oxide fractions. However, the comparison between these two extractions could be useful for showing differences between fractions with different ease of reduction and therefore different potential availability.

In the GAR soil, there was a relatively small concentration of Cu_{ox} in comparison with the Cu_{asc-ox}. In this soil, Cu associated with metal oxides may be bound mainly to the crystalline forms of these compounds, which are less reactive and less easily reducible than the amorphous forms and are associated with fewer available metals. Conversely, in the treatments of soil CUA, there was a relatively large proportion of Cu associated with the amorphous oxides in this soil. The Cu_{asc-ox} concentration did not vary significantly among the different treatments of organic amendments. However, in CUA60M, the concentration of Cu_{ox} was significantly smaller ($P < 0.05$), because of the re distribution of Cu from this fraction to the organic-bound fraction when the manure amendment was applied.

As for Zn (Table 6), there was also a small amount of this metal extracted with oxalate (Zn_{ox}) in GAR treatments. In the CUA soil there was a greater concentration of Zn_{ox}, and therefore a greater availability. The concentrations of Zn in ascorbate–oxalate extracts (Zn_{asc-ox}) did not vary among the different treatments. However, there was a significantly greater amount of Zn_{ox} when manure was applied to GAR soil ($P < 0.05$), which probably resulted from the high pH of this amendment and resulted in an increase in Zn sorption on amorphous oxides.

Conclusions

The mine soils studied contained had large levels of metal contamination and poor fertility, which limit the establishment of the vegetation cover and their agricultural and forest use. The application of sheep and horse manure and pine bark amendments on these contaminated soils resulted in an increase in organic matter content and modified pH and other soil properties. These modifications affected soil fertility and metal bioavailability for plants.

The pine bark amendment did not significantly increase the nutrient content of soils, therefore this organic material should not promote plant growth. However, this amendment increased the metal availability probably because of lower pH and the formation of soluble organo-metallic complexes. If this metal mobilization does not result in a greater metal uptake by plants, it could increase the risk of metal leaching into groundwater and the toxicity for other soil organisms, such as microflora.

The manure amendment reduced Cu and Zn bioavailability because of its large pH and degree of humification and maturity of its organic matter. The manure amendment not only reduced metal toxicity and provided organic matter and a better pH, but also raised the CEC and improved the nutrient status of the soil, which could promote plant growth and permit the re-establishment of vegetation. The application of this manure as a soil amendment at doses of 60 t ha^{-1} of dry organic matter (215 t ha^{-1} of fresh matter) and at a cost up to US \$8000 ha^{-1} appears to be a useful and cost-effective strategy for the restoration of contaminated mine soils and the establishment of phyto-remediation technologies.

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